A STUDY OF THE EFFECT OF GROWTH SUBSTANCES ON FRUIT-SETTING OF THE MANGO

By

THOMAS TRACY STURROCK

A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL OF
THE UNIVERSITY OF FLORIDA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE
DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA
January, 1961

ACKNOWLEDGEMENTS

The author wishes to acknowledge the many sources of aid received by him in the course of this research. The many members of the faculty of the University of Florida and the staff of the Florida Agricultural Experiment Station who have been beneficial in supplying guidance and aid in this research are too numerous to mention. The many graduate students and Station employees who have offered suggestions and helping hands throughout this work have been of incalculable aid.

Sincere appreciation is expressed to the following members of the Supervisory Committee which directed this research:

Dr. H. S. Wolfe, Professor of Fruit Crops, Chairman of this Supervisory Committee, has been a constant source of guidance and inspiration. In addition, he has contributed 20 years of horticultural instruction and guidance to the author.

Dr. R. H. Biggs, Assistant Biochemist, Department of Fruit Crops, Florida Agricultural Experiment Station, Co-Chairman of this Supervisory Committee, has been a most valuable advisor throughout this research with special regard to biochemical and plant physiological considerations.

Dr. Yoneo Sagawa, Assistant Professor of Botany, has been a source of information and instruction in cytological techniques which were instrumental in the conduct of this research.

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Dr. T. W. Young, Horticulturist, Florida Agricultural Station, who has had many years of both technical and practical experience with mangos, has been a valuable source of advice throughout this work.

Dr. D. S. Anthony, Associate Professor of Chemistry, has been helpful with counsel on chemical matters.

In addition to the above members of this Supervisory Committee, the following persons have been of outstanding help in this research:

Dr. A. E. Brandt, Head of the Statistical Section, Florida Agricultural Experiment Station, has been a very valuable consultant in statistical matters. Through his advice and counsel the statistical analyses of this research were handled in the indicated manner. His aid in setting up the statistics and interpreting the results as well as his criticism of this dissertation from a statistical point of view have been a great asset.

Dr. John W. Sites, formerly Head of the Department of Fruit Crops and presently Associate Director of the Florida Agricultural Experiment Station, has maintained continued interest in this problem. In addition his many actions in behalf of the author in arranging facilities for this work and financial aid through assistantships and fellowships will always be held in high personal remembrance.

Dr. A. H. Krezdorn, Head of the Department of Fruit Crops, has continued the interest in and aid of this project which was initiated by his predecessor.

The author wishes to express a special acknowledgement to the help given by his father, David Sturrock of West Palm Beach, Florida,

whose continued encouragement and consultation during the course of this work have been very beneficial. Without the many hours which he spent in maintenance of the experimental grove and in helping with the application of treatments and recording of data this research could not have been accomplished.

Finally, but by no means least, the author expresses his profound gratitude to his wife, Jeanne, and their five children who have been a source of comfort and inspiration during the trying months of this work. By their giving up their established way of life and enduring the rigors of Spartan college living to be with him, it has been possible for him to maintain his morale and presence of mind so that this research could be accomplished.

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CHAPTER I

INTRODUCTION

A major problem in the commercial production of the mango (*Mangifera indica* L.) is its poor fruitfulness. There are many reasons proposed for this lack of fruiting. In India the condition of alternate bearing exists. The primary cause for this is the formation of blossom-buds only in years in which bearing is light. In Florida there is no problem with blossom-bud differentiation as abundant blossoms are produced almost every year on most varieties. In contrast, the problem here is poor fruit-set.

Mango varieties fall into two general types: those developing only one embryo per seed (monoembryonic) and those developing more than one embryo per seed (polyembryonic). The polyembryonic types have been observed to bear fruit more consistently than the monoembryonic types.

Much work has been done with other commercial fruits (e.g., apples, cherries, and peaches) to increase their fruit-set by treatments with growth substances.

The objective of this research was to find causes of this poor fruit-set of mango. The identification of these causes might then lead to practical controls of this problem. A study was made of the effect on fruit-set of the mango by the application of various of these growth substances at different times and at different concentrations.

A study was also made of the monoembryonic and polyembryonic types to try to establish the basis of the difference in their fruiting. Histological examinations of the developing fruit were made to determine if there was any difficulty with the fertilization mechanism. Analysis of the growth substance content was made to determine if there was any really apparent difference in the regulator chemical make-up between these two types of mangos.

In addition to these main facets of this investigation, several collateral experiments were conducted to determine whether lack of nutrients or competition between the many flowers might be a factor in reducing mango fruit-set.

CHAPTER II

LITERATURE REVIEW

Much has been written and said about the mango since its first historical record in India over 4,000 years ago (10). It is now found throughout the tropical world, with different varieties developed and adapted to various regions. The mango has been described as "king of fruits" (19) and "the choicest fruit of Hindustan" (9, 18, 51), because this fruit plays such an important part in the diet and culture of the natives of the areas in which it is grown. Since the first successful introduction of grafted mangos in 1889 (51, 74), the mango industry in Florida has grown until at the present time it makes a real contribution to the economy of the state. Other commercial production areas for the mango are found in India, the Philippine Islands, Indonesia, Burma, Malaya, Thailand, Central America, northern South America, and the West Indies.

The literature regarding the mango, both technical and popular, covers all aspects of its history, varieties, uses, culture, and botany. Since this research is primarily concerned with the problems of fruit-setting, the literature review will deal with the general areas of mango culture only insofar as they have particular bearing on the problem at hand.

While the mango fruit is highly prized as both a staple and a delicacy, there are many problems arising in its culture. The

primary problem with most commercial varieties is erratic fruiting. In recent years (since about 1900) many authors have commented on this erratic fruiting. Woodrow writing from India in 1904 (77) stated: "If the hot cloudy weather which accompanies thunder-storms and turns milk sour occurs while the trees are in blossom, the flowers mostly wither and fall off without fertilization, and in consequence the crop is scanty." Ruehle and Ledin in 1955 from Florida (57) wrote: "One of the worst defects of the mango is the pronounced tendency to produce a light crop even though the tree flowers profusely." Spencer and Kennard in 1956 from Puerto Rico (62) expressed the situation well when they said: "The mango . . . characteristically produces relatively few mature fruits from masses of flowers." These and many similar remarks characterize the undependable bearing of this most desirable tree.

Several suggestions for this light or erratic bearing have been advanced by workers over the past sixty years. These can be divided into two categories: (1) environmental factors influencing fruit-set, and (2) internal factors associated with natural fruit-set of mangos.

Environmental Factors Influencing Fruit-Set

Site

The mango is found throughout the tropical regions of the world, and extends into the subtropics in regions not subjected to low temperature in winter (57, 67). Temperatures below 32° F. may

kill very young trees, injure new flushes of growth, and damage flower clusters (57).

Annual precipitation in mango-growing parts of the world varies from 1.3 inches to over 80 inches (48). A definite alternation of wet and dry seasons should exist for good fruitfulness: a wet season to encourage vegetative growth and a dry season to arrest vegetative growth and encourage formation of blossom buds (57).

While soil requirements for mango growing are variable, the tree will grow on almost any well-drained soil (65). It grows best on deep loam containing some humus, but will flourish on light acid sands as well as on alkaline limestone soils if properly fertilized (57).

Water drainage and air drainage are important. Although mango trees have been known to survive after being flooded for six weeks (57), good water drainage should be considered in selecting the proper site for a mango planting. Air drainage is important for prevention of injury to trees due to low winter temperatures, as cold air settles in low pockets causing damage (14).

An environment which will produce healthy growing trees yet allow proper blossom-bud differentiation and flower development is a necessity for good fruit-set. Environmental factors which limit these conditions are therefore limiting to fruitfulness. In regions where mangos are grown, slight changes in environment (other than low winter temperatures) usually do not greatly affect fruit production.

Cultural Practices

In general the mango requires about the same practices as other orchard trees (7, 14, 59). Selection of the site should include consideration of soil, irrigation, water drainage, air drainage, and minimum winter temperatures as previously mentioned (37, 57, 64, 68). These factors contribute to maintaining healthy trees. A healthy growing condition of the tree itself is a necessity before fruit can be expected.

More specifically, planting distances can be a limiting factor in fruit bearing. If the trees are planted too close together, the outer canopy of branches of adjoining trees will overlap. This will cut down on air movement, increase the difficulty of spraying, and promote the spread of anthracnose (54). This will also reduce fruit-set by shading of overlapping branches, pushing bearing surfaces up and eliminating peripheral twigs as potential bearers. Planting distances from 30 to 45 feet apart are recommended, depending upon the variety and the type of soil (18, 40, 54).

Proper fertilization practices for bearing mango trees have been the subject of continuing research (27, 28, 29, 30, 31, 32, 39, 40, 51, 53, 55, 57, 64, 79, 81, 82, 83, 84, 85). In general, most investigators agree in recommending applications of nitrogen about the time of or just preceding blossom-panicle elongation. An application of a complete fertilizer is recommended one to two months later. Another application of complete fertilizer is recommended to be applied to the trees after the fruit has been harvested. This application

helps produce strong vegetative growth which will develop strong blossom-buds for a potential crop the following year.

The quantity and analysis of fertilizers for each application are far from being agreed upon by researchers. The size of the trees, their bearing history, and the type of soil are factors to be considered in making recommendations. The mango is a vigorous growing tree, drawing well on the major and minor nutrient elements when they are available. Deficiency symptoms of mango have been determined for various minor elements (41, 61). In connection with fruiting, Young (79, 81, 82, 84) has found no correlation between fertilizer treatments and yields over a series of trials. He did find that the yield of one variety (Kent) responded to increases in nitrogen fertilizer (84, 85). This lack of correlation is further evidenced by many observations (40, 50, 57, 62, 63) of mango trees in vigorous growth which blossom profusely yet fail to set an acceptable crop of fruit.

Pruning is recommended in the case of very large trees which require a reduction in size to eliminate crowding. This allows better control of diseases and insects and makes harvesting of the fruit easier. The removal of dead wood, repairing of cold- or wind-damaged trees, and the rejuvenation of old or weak trees require some pruning. An annual pruning is not recommended (6, 40, 57, 66).

Windbreaks are not recommended for mango groves in Florida. Since the mango tree is fairly large and resistant to wind damage, they are not necessary. Windbreaks have been shown to be detrimental to fruiting of mangos by reducing air movement, thus promoting the

development of and damage by anthracnose (57). However, windbreaks are recommended in India for the conventional reasons (12).

In summation of cultural recommendations for the mango, the usual cultural practices for orchard fruits are followed with few exceptions. Since this tree is evergreen it requires a period of restrained vegetative growth to initiate the differentiation of flowers in large quantities for a potentially good crop. In general, this is brought about by the normal reduction of temperatures in the fall of the year, together with a decrease in soil moisture.

Since healthy trees with profuse bloom have been observed on many occasions to set a very light crop or no crop, cultural practices are not deemed to have a deciding effect on fruit-set other than maintaining healthy growing conditions.

Insect Pests

Many insects have been found using the mango as a host. Although Merrill (44) reports over twenty species of scales alone which have been found on mango, only three of these are often found to be troublesome to the extent of reducing tree vitality (57). Red-banded thrips (Selenothrips rubrocinctus Giard.) and avocado red mite (Paratetranychus yothersi McG.) cause severe defoliation of mango trees when populations are allowed to become heavy (57). If the vitality of the tree is reduced it is reasonable to expect that the blossom-bud differentiation will also be reduced, thus reducing the potential crop.

Some damage to mango blossom panicles by various beetles and lepidopterous larvae has been reported (45, 49, 75).

Wolfenbarger in 1956 (75) reported Florida flower thrips (Frankliniella cephalica D. L. Crawford) to be common in mango bloom; however, in 1957 (76) he reported: "Fruit-set was not related to thrips abundance nor to insecticidal applications."

Three species of Idiocerus (niveosparsus, atkinsonii, and clypealis Leth.) known as "mango hopper" do considerable damage to blossom panicles in India and the Philippine Islands (2, 12, 49, 72, 73). They can be controlled with contact oil sprays, sulphur dust, and DDT (2, 12, 49). These insects, while doing considerable damage to the potential crop in India and the Philippine Islands, have not been reported from other parts of the world.

The effects of insects on mango yields are largely indirect. Since they are rather easily controlled, they are limiting factors in fruit-set only where poor orchard practices are followed.

Diseases

Anthracnose (Colletotrichum gloeosporioides Penz.) is the one major disease causing appreciable losses to the mango in all areas in which it is grown (57). It is an extremely important factor in lowering the grade and quality of mango fruit, and in most varieties it contributes to lack of fruitfulness in some seasons (57). Fortunately, with our present-day knowledge of its etiology and our modern fungicides, this disease can be controlled effectively (56).

Mango scab (Elsinoe mangiferae Bit. and Jenkins) has also been found to infect the mango but not to the extent of anthracnose (54). Usually spray schedules for the control of anthracnose will also control scab in bearing groves.

Powdery mildew (Oidium sp.) has been reported to cause blossom infection in both Florida and India (12, 57). In severe attacks the entire blossom panicle may be involved and the fruit fail to set.

Rusts and epiphytic algae are found occasionally on mango trees which are in a neglected condition. However, with proper cultural practices, such as removal of dead wood and proper fungicidal spraying, these diseases are also readily controlled.

Diseases such as anthracnose which cause a complete breakdown of the flower panicle definitely are factors in reducing the fruit-set of mango. Nevertheless, there are many instances when the mango has failed to set a good crop although adequate controls for disease were provided.

Internal Factors Associated with Natural Fruit-Set

Imperfect Flowers

The inflorescence of the mango is made up of both staminate and perfect flowers (12, 18, 25, 50, 57). The percentage of perfect flowers per panicle is variable (1.25 to 87.5 per cent) depending upon the variety and the year (8, 18, 30, 31, 32, 46). The total number of flowers per panicle depends on the size of the panicle itself, which in turn depends on the vigor of the shoot and the degree of flower induction. This number has been estimated to range from "a few hundred" to "over 9,000" (18, 46, 57).

Ledin and Malcolm (30, 31, 32) observed that varieties with low percentages of perfect flowers consistently produced low yields while varieties which generally produced satisfactory yields in most years

had a higher percentage of perfect flowers. However, as Hayes (18) pointed out and as can be determined from the aforementioned figures, even with the lowest percentages of perfect flowers there are still more than enough to produce an excellent crop. While a small number of perfect flowers would reduce the possibility of a very large set, there are still adequate perfect flowers to produce more fruits than are usually matured on a single panicle.

Poor Pollination

Much speculation has been made that poor pollination is a causal factor for poor mango fruit-set. It had been observed that certain varieties of mango seemed to fruit better in mixed plantings than when planted in solid blocks (18, 37, 58). After nine years of controlled testing, however, there was no valid evidence to support this assumption (32).

Popenoe (50) showed that pollen did not germinate below 55° F., and that best germination was not achieved until the temperature was at least 75° F. Young (80) found similar results, and in further tests (81, 82, 84) concluded that exposure to low temperatures (provided that freezing did not occur) had no adverse effect on the eventual germination of pollen when the optimum temperature was again restored.

Popenoe (50) has indicated that pollination is a requirement for fruit-set, but that no appreciable increase in fruit-set has resulted from applying an abundance of pollen. He further stated that "apparently the difficulty is not due to any morphological defect in the

pollen or to defects in the mechanism of pollination." Young (78), however, reported an increase in fruit-set on the Haden variety with an increase in pollen applied by hand. Juliano and Cuevas (25) working with the Pico variety in the Philippine Islands reported that pollination was necessary for the formation of fruits with viable seeds.

The mango has been reported to have a multi-nucleate endosperm arising from the union of the endosperm nucleus of the embryo sac and one of the sperm cells from the pollen tube (23, 24, 25). Belling reported that the majority of the fruits under 7 mm. in length that he examined showed no multi-nucleate endosperm, while all fruits over 7 mm. in length showed this condition to exist. From this he concluded that fertilization occurred before the fruit reached this size (4). Young (78) pointed out that merely the presence of a multi-nucleate endosperm is not positive proof that fertilization has occurred. Endosperm is known to develop apomictically in some plants from just the union of the two polar nuclei (21, 43).

As previously mentioned, some varieties of mangos have several embryos (polyembryonic). Except for the one gametic embryo, the rest of these are of a vegetative nature, developing from the nucellus. Johansen (21) in discussing nucellar embryos placed the mango in two different categories. In the first he included species in which fertilization and triple fusion must first occur and endosperm development start before nucellar cells could be established as adventitious embryos. In this he agreed with Maheshwari (43). Johansen also listed the Strawberry variety of mango as being in the second category, being able to develop nucellar embryos without first being fertilized,

and cited Juliano (23) as his authority for this statement. Examination of Juliano's article, however, does not indicate that this conclusion was reached by him.

Spencer and Kennard (63) reported finding a condition of limited receptivity of the stigma precluding the germination of pollen, thus making pollination ineffective at times.

Monoembryony

Many varieties of mangos have been shown to produce more than one seedling per seed, a condition known as "polyembryony" (20, 45). Beiling (4), working with the No. 11 variety in Florida, found that the development of several seedlings resulted from the production of nucellar embryos. This same conclusion was reached by Juliano (23, 24) and by Juliano and Cuevas (25), working with the Pico, Carabao, and Strawberry varieties in the Philippine Islands. These workers observed a very high percentage of degeneration of gametic embryos, with subsequent development of the nucellar type.

Observations of the author and others (78) indicate that the varieties which are polyembryonic are more prolific bearers than are varieties which are monoembryonic. This might be explained on the basis of the number of embryos present. In the cases of the monoembryonic types, when abortion occurs there is no embryo to support further development of the fruit. In the case of the polyembryonic types, when abortion of the gametic embryo occurs the nucellar embryos are present to supply factors needed for the development of the fruits. The condition also might exist that the mango could develop nucellar

embryos without fertilization as implied by Johansen (21). Therefore, the chance of having poor fruit-set is greater in the case of monoembryony.

Those who have studied mango varieties from different parts of the world have shown that the polyembryonic varieties of desirable dessert quality are chiefly those which have originated in the Philippine Islands, and which in turn came from southeast Asia. Some of the seedling races (e.g., No. 11, Turpentine, Apple, Peach) are of other than southeast Asia origin while being polyembryonic. The Indian mangos are chiefly monoembryonic (53), but some polyembryonic varieties are known to be native to that country (42).

Fertilization Defects

Juliano (23, 24) and Juliano and Cuevas (25) found that the zygote of the mango does not divide soon after fertilization but may remain dormant for some time while the endosperm develops. Maheshwari (42) says that this delay in development of the zygote is longer, perhaps, than in other angiosperms. Juliano and Cuevas also found evidence of degeneration of zygotes. Since the varieties with which they were working were of the polyembryonic type, nucellar embryos were formed from the nucellar layer, possibly stimulated by a developing endosperm. Upon degeneration of the zygote, polyembryonic seeds containing only nucellar embryos developed. The fact that these fruits were carried to maturity might explain the observation that polyembryonic types are better bearers than are the monoembryonic types.

Young (78), working with a monoembryonic variety (Haden) in Florida, concluded that a major cause of unfruitfulness was a breakdown of the egg mechanism. He also found evidence of embryo abortion after initiation of fruit development.

Blossom-Bud Differentiation

The proper formation of blossom buds, of course, is an essential for good fruit-set (see "Cultural Practices"). This is exemplified by alternate bearing which is the principal production problem of the mango in India. This is well explained by Bajwa et al. (3) when they wrote that " . . . alternate bearing in the case of the mango . . . is based on the fact that an antagonism exists between vegetative growth and flowering. It means that shoots which bear panicles do not make any vegetative growth during the season of flowering and thus remain barren the next year while the shoots which do not flower make vegetative growth early in the season and are the ones to bear fruit the next year." As stated previously, lack of differentiation of blossom buds is not usually a factor in low yield of mangos in Florida.

Chemical Modification of Fruit-Set

General

In 1936 Gustafson (16) showed that tomato fruit could be set in the absence of pollen by the application of certain growth-promoting substances on the cut flower stigma. Since that time improved techniques have been developed whereby growth-promoting substances may be introduced into plants by more effective means such as spraying the

flowers and foliage, watering the roots, applying aerosol mist on the foliage, and placing the materials into the transpiration stream of the plant by various mechanical methods (1, 13, 17, 33, 69).

Parthenocarpic fruit-set has been promoted on a number of different plants (tomato, holly, grape, fig), and at the present time this is in many instances an established horticultural practice (1, 16, 17, 33, 60, 69). These parthenocarpic fruits are essentially seedless, which may be either an advantage or a disadvantage depending on the fruit and its proposed use (17).

In addition to parthenocarpic fruit production, it has been shown that certain growth-promoting chemicals will aid in normal pollination and retention of fruit on the tree after it has been set (e.g., apple, pear, apricot, and citrus). Luckwill (34, 35, 36), working with apples in England, showed that during the development of the fruit natural hormones were produced in succession from the ovules, the endosperm, and the embryo. Between the times of production by these individual tissues there was a deficiency of the natural hormones. These periods of low hormone production were associated with periods of high fruit-drop. When hormones were applied during these periods of low natural hormone production, the fruit-drop was greatly reduced.

In addition to promotion of fruit-set and fruit-retention by chemical means, fruit-thinning has also been accomplished (e.g., apples and peaches). In some cases the same compound applied at different concentrations and at different times may be used for either fruit-setting or fruit-thinning (33).

The relationship between low hormone level and abscission has been shown by Biggs and Leopold (5). Abscission is promoted when the hormone level in the plant decreases. Abscission is prevented by keeping the hormone level in the plant high. The fruit is lost if the natural hormone production of the organ drops to a level where abscission is encouraged. This drop in natural hormone production may result from a change in the productive tissues, as shown by Luckwill's work (34, 35, 36), or from the death of the tissue. High fruit-drop can be prevented in some plants by applying synthetic hormones during these periods of low natural hormone production.

On Mango

Several unsuccessful attempts to use various growth substances on the mango have been recorded. Lynch (38) in 1940 tried naphthaleneacetic acid, indolebutyric acid, indolepropionic acid, and indoleacetic acid on the blossom panicles. Since the results of this experiment have not been published it is assumed that they were negative. Ledin (28) in 1952 tried sodium naphthalene acetate and maleic hydrazide in an attempt to induce vegetative dormancy prior to floral initiation. These tests were inconclusive. Kennard and Winters (26) in 1956 applied 2,4,5-trichlorophenoxypropionic acid on mango blossoms three weeks after bloom. They reported that maturity was hastened and fruit-size was reduced, but made no mention of increased fruit-set.

In 1949 Venkataratnam (70) applied 10 ppm. beta-naphthoxyacetic acid in water to emasculated and bagged pistillate blossoms. While all fruit dropped at "marble size," an increase in initial fruit-set by about 100 per cent over the non-sprayed controls was noted. This

indicated that mango fruit could start development without pollination and that this initiation could be helped with the application of auxins.

The only other report on record of increased fruit-set of mango by using growth substances is that of Gokhale and Kanitkar (15) from India in 1951. By using 25 ppm. naphthaleneacetic acid or 2,4-dichlorophenoxyacetic acid when the fruit was from one-half inch to one inch in diameter they were able to increase the yield at harvest by 17 to 23 per cent. Counts made by Wagle (71) showed that by the time the fruit was from one to one and a half inches in diameter, only 0.25 per cent of the perfect flowers had developed as fruit. Mukherjee (46) reported that usually only 0.1 to 0.25 per cent of the perfect flowers develop into mature fruit. Therefore, it is evident that a great number of developing fruit had fallen by the time these sprays were applied. Nevertheless, it is indeed interesting that the tendency for late fruit-drop was lessened.

CHAPTER III

CHEMICAL SCREENING, 1959

Various chemical growth substances have been shown to be effective in causing the setting of fruit on many plants. These have caused the setting of parthenocarpic fruit as well as the stimulation of gametic embryo growth, preventing the premature dropping of fruit.

In order to screen growth substances for possible activity in increasing mango fruit-set, various chemicals (see Table 1) were tested on mango flowers during the 1959 blooming season.

Materials and Methods

Each of 28 chemicals as listed in Table 1 was used in three different concentrations: 1×10^{-3} molar, 1×10^{-4} molar, and 1×10^{-5} molar. These were coded as "-3," "-4," and "-5" respectively for ease of labeling. A 0.05 per cent solution of Tide detergent was used in the solution to serve as a wetting agent. All solutions were adjusted to pH 7.0.

The chemicals were sprayed on the flower panicles with the use of a hand-operated atomizer. Each flower panicle was thoroughly wet to run-off.

So that time of application could be studied, some of the panicles were sprayed "early" (just as the first blossoms on the panicles were starting to open), some were sprayed "late" (as the

TABLE 1

LIST OF CHEMICALS USED, 1959

Code	Chemical
A	o-Chlorophenoxyacetic acid
B	p-Chlorophenoxyacetic acid
C	o-Chlorophenoxyisobutyric acid
D	p-Chlorophenoxyisobutyric acid
E	p-Chlorophenoxypropionic acid
F	2,4-Dichlorophenoxyacetic acid
G	3-Indoleacetic acid
H	3-Indoleacetic acid, ethyl ester
I	3-Indolebutyric acid
J	3-Indolepropionic acid
K	1-Naphthaleneacetamide
L	alpha-Naphthaleneacetic acid
M	alpha-Naphthaleneacetonitrile
N	beta-Naphthoxyacetic acid
O	Phenoxyacetic acid
P	alpha-Phenyl, N-butyric acid
Q	gamma-Phenyl, N-butyric acid
R	(Control--0.05 per cent Tide solution)
S	trans-Cinnamic acid
T	Coumarin
U	2,4-Dinitroanisole
V	2,4-Dinitrophenol
W	Maleic hydrazide
X	2,3,5-Triiodobenzoic acid
Y	Potassium gibberellate
Z	Kinetin
AA	2,4,5-Trichlorophenoxyacetic acid, amyl ester
BB	2 (2,4,5-Trichlorophenoxy)-propionic acid
CC	2,4,5-Trichlorophenoxyacetic acid
CONT	(Unsprayed control)

panicles were almost finished blooming--three weeks after the "early" spray), and some were sprayed "both" (sprayed at both of the above times).

The panicles for this screening were selected at random, the only restriction being that all nine panicles for a given chemical ("early," "both," and "late" for each of the three concentrations) would appear on the same tree. Each set of nine panicles was repeated five times to give a total of 45 panicles receiving each chemical. Ten panicles for "early," ten for "late," and ten for "both" were used for the Tide control. Thirty unsprayed panicles were marked for comparison purposes.

At intervals, the fruits remaining on each panicle were counted and recorded. These counts were used to determine the relative effectiveness of the various treatments.

The field work for this research was carried out in the author's grove in West Palm Beach, Florida. This grove consists of a solid planting of the Edward variety (a monoembryonic type). It is planted on a Lakewood type soil and had better than average soil and air drainage. Throughout the experiment the grove was kept in excellent cultural condition.

The laboratory work for this investigation was conducted with the aid of the facilities of the Department of Fruit Crops, Florida Agricultural Experiment Station, Gainesville, Florida.

Results

The first count of fruit remaining on the panicles was made on February 28, 1959. This was four weeks after the early spray and one week after the late spray. The second count was made two weeks later on March 14, 1959.

Following the second count and before the third count, which was made two weeks later, on March 30, 1959, unusually strong winds occurred in the experimental grove. These winds of more than 50 miles per hour lasted over a period of three days. As a result a heavy drop in fruit occurred, and it could not be determined how much of this fruit would have fallen due to normal circumstances. It was assumed that fruit-set had been reduced. Therefore, to gain information on the effectiveness of the treatments the existing data were used as the best available for this purpose. A ratio of the number of fruit left on the panicle at the second count to the number of fruit at the first count was used as this index.

The results of these first two counts are listed in Tables 2, 3, and 4. The numbers of fruits following the second count were quite low, therefore, these later counts are not included. The effect of time of spraying in Table 3 shows no significant difference on fruit-set. The effect of concentration of compound in Table 4 shows no significant difference to exist among effects of concentrations in general. These results were statistically analysed by the use of Factorial Chi Square.

TABLE 2

EFFECT OF CHEMICALS, 1959

(Numbers are total fruit remaining on 45 panicles.)

Treatments	First (T)	Second (r)	Ratio (r/T)	Rank	(r^2/T)
A	489	79	0.162	25	12.76
B	480	93	0.194	12	18.02
C	530	99	0.187	17	18.49
D	395	73	0.185	18	13.49
E	475	79	0.166	24	13.14
F	429	83	0.193	13	16.06
G	577	99	0.172	22	16.99
H	551	129	0.234	2	30.20
I	435	74	0.170	23	12.59
J	409	66	0.161	26	10.65
K	409	77	0.188	16	14.50
L	251	27	0.108	28	2.90
M	374	87	0.233	3	20.24
N	723	160	0.221	7	35.41
O	539	121	0.224	5	27.16
P	716	131	0.183	19	23.97
Q	405	92	0.227	4	20.90
S	309	65	0.210	10	13.67
T	400	72	0.180	21	12.96
U	444	95	0.214	9	20.33
V	484	77	0.159	27	12.25
W	336	74	0.220	8	16.30
X	465	88	0.189	15	16.65
Y	343	81	0.236	1	19.13
Z	455	102	0.224	6	12.87
AA	132	25	0.189	14	4.73
BB	169	33	0.195	11	6.44
CC	150	27	0.180	20	4.86
Total	11,874	2,308			457.66
Controls					
R	357	54	0.151		
CONT	339	73	0.215		

Minus correction factor = $\frac{(2,308)^2}{11,874}$ = 448.62

9.04

Chi Square factor = $\frac{(11,874)^2}{(2,308)(9,566)}$ = 6.39

Chi Square = (9.04)(6.39) = 57.77

Chi Square for 27 D/F (0.001) = 55.476

Therefore, a very highly significant difference exists.

TABLE 3

EFFECT OF TIME OF SPRAYING, 1959

(Numbers are total fruit remaining on 420 panicles.)

Sprayed	First (T)	Second (r)	Ratio (r/T)	Rank	(r^2/T)
Early	4,237	813	0.192	2	156.00
Both	3,890	801	0.206	1	164.94
Late	3,747	694	0.185	3	128.54
Total	11,874	2,308			449.48
Minus correction factor = $\frac{(2,308)^2}{11,874}$				=	448.62
					0.86
Chi Square factor = $\frac{(11,874)^2}{(2,308)(9,566)}$				=	6.39

Chi Square = (0.86)(6.39) = 5.50

Chi Square for 2 D/F (0.05) = 5.99

Therefore, no significant difference exists.

TABLE 4

EFFECT OF CONCENTRATION OF SPRAY, 1959

(Numbers are total fruit remaining on 420 panicles.)

Conc.	First (T)	Second (r)	Ratio (r/T)	Rank	(r^2/T)
"-5"	4,101	829	0.202	1	167.58
"-4"	3,968	780	0.197	2	153.33
"-3"	3,805	699	0.184	3	128.41
Total	11,874	2,308			449.32
Minus correction factor = $\frac{(2,308)^2}{11,874}$				=	448.62
					0.70
Chi Square factor = $\frac{(11,874)^2}{(2,308)(9,566)}$				=	6.39

Chi Square = (0.70)(6.39) = 4.47

Chi Square for 2 D/F (0.05) = 5.99

Therefore, no significant difference exists.

Discussion

Since this experiment was designed to screen known growth substances in respect to their effect on mango fruit-set, some criterion had to be established for a means of judging the results. The ratio of the number of fruit remaining on the second count to the number of fruit on the first count was deemed the best method available.

Admittedly this was a test to screen a good number of available compounds. Therefore, to test this number of materials it was necessary to sacrifice replications, resulting in a large internal-error factor. This was why the 1960 experiment was designed to correct this situation.

From the rank column of Table 2 it can be seen that eight chemicals ranked better in this ratio of the second count to the first (r/T) than did the unsprayed control. Only one chemical (L) was poorer in this respect than was the Tide-sprayed control.

It will be noted that these eight chemicals which ranked high in this ratio are not necessarily the same chemicals which had a high number of fruits in the first count. For example, the No. 1 ranking chemical in ratio was Y, but it would be No. 21 in rank as far as totals for the first count are concerned. It is conceivable that the sprays could have had an effect in reducing the total number of fruit on the panicles, but once this number was reduced there was little subsequent drop. This apparently was the case with Y (potassium gibberellate) as the first count was rather low, but a smaller drop was experienced by the time of the second count giving a higher ratio than for the others.

On the basis of this ratio method of selection, eight chemicals were deemed to have a ranking better than the unsprayed control. They are as follows:

Rank	Code	Chemical	Ratio
1	Y	Potassium gibberellate	0.236
2	H	3-Indoleacetic acid, ethyl ester	0.234
3	M	alpha-Naphthaleneacetonitrile	0.233
4	Q	gamma-Phenyl, N-butyric acid	0.227
5	O	Phenoxyacetic acid	0.224
6	Z	Kinetin	0.224
7	N	beta-Naphthoxyacetic acid	0.221
8	W	Maleic hydrazide	0.220
9	CONT	Unsprayed control	0.215

Aside from maleic hydrazide, which had the ratio approaching the control, all the other compounds above the unsprayed control are known plant growth promotors.

Of these chemicals, kinetin was not available in sufficient quantity to allow it to be used in the expanded second year test. To reduce the number of treatments so that more replications could be used, it was decided to limit the second year test to six chemicals at two concentrations each. Therefore, maleic hydrazide, which was the lowest ranking chemical on the list, was dropped.

Summary and Conclusions

Based on these experimental results, the remaining six chemicals as listed above were selected for use in the 1960 spraying experiment.

CHAPTER IV

CHEMICAL SPRAYING, 1960

With the information gleaned from the preliminary screening test in 1959 of chemicals for their effectiveness in increasing mango fruit-set, a more precise experiment was designed to test formulations which had shown some promise. In planning this phase of the investigation, an attempt was made to follow a practical grove-spraying procedure more closely than could be done with the hand-atomizer method.

Materials and Methods

The following chemicals were used in the 1960 spraying program. They are listed with their code designations (the same as used for them in the 1959 test). These codes were used for labeling and recording data to simplify the writing of the long technical names of the compounds.

Code	Chemical
H	3-Indoleacetic acid, ethyl ester
M	alpha-Naphthaleneacetonitrile
N	beta-Naphthoxyacetic acid
O	Phenoxyacetic acid
Q	gamma-Phenyl, N-butyric acid
Y	Potassium gibberellate

Two concentrations of 1×10^{-4} molar and 1×10^{-5} molar were used for each compound. These concentrations were coded as "-4" and "-5," respectively. The concentration code was used in combination with the compound code (e.g., H-4 stands for 3-indoleacetic acid, ethyl ester at 1×10^{-4} molar concentration).

The diluted sprays were placed in a Hudson 1.5 gallon pump sprayer. DuPont Spreader Sticker at the rate of 12 drops per gallon was added. This spray was applied uniformly to the entire tree so as to wet the blossom panicles and the leaves to the point of run-off. Approximately 4 liters of solution were used per tree.

Four trees were sprayed with each treatment. Also, four trees were sprayed with a similar concentration of DuPont Spreader Sticker only in water, and four unsprayed trees were tagged for comparison purposes as a further control. The trees for each treatment were picked at random in the grove. The only restriction was that an attempt was made to balance the treatments as to size of trees. Where a tree which was larger than average was used for a particular treatment another tree which was smaller than average was included to offset any advantage of size or vigor that the first would give to that particular test.

Previous work (8) has shown that the majority of the perfect flowers are produced during the first ten days of a panicle's period of opening. For this reason an attempt was made to apply the treatments during this period of blossom development. While there were some early blossoms and some late ones, the general opening of the mass of blossoms in the grove was used as an index for the time of application. This general opening occurred about January 15, 1960. The sprays were applied over a three-day period, January 21-23, 1960.

The number of fruits on 40 panicles on each tree was determined at designated times during the growing season. These counts were used to measure the relative effectiveness of the treatments. The fruit on

40 panicles per tree were counted at each period of recording. To insure a uniform sampling from all parts of the tree (high, middle, and low) and from all exposures (NE, NW, SW, and SE), the 40 panicles were distributed evenly among the quadrants of the tree. Of the ten panicles in each quadrant, three were in the upper third, four were in the middle third, and three were in the lower third of the tree. The same panicles were checked at each period of data recording. When all the fruit had fallen from a particular panicle, the label was removed so as to avoid confusion and to make the task of finding the desired labels easier.

The field work for this experiment was carried out in the same grove in West Palm Beach, Florida, as was the 1959 screening experiment (Chapter III).

Results

The first counts were made on February 18-19, 1960 (four weeks after spray application). Subsequent counts were made at two-week intervals until maturity. The total counts for the four trees of each treatment were combined, and this figure was used to compare the relative effectiveness of each treatment with that of the controls.

The results from these counts are recorded in Table 5. Analyses of these results are presented in Tables 6 through 10. Graphs showing the relationship of the results of treatments M-4, N-4, and Y-4 in regard to the unsprayed control and the spreader-sticker control are shown in Figures 1, 2, and 3 respectively.

TABLE 5

RESULT OF CHEMICAL SPRAYING, 1960

(Numbers are total fruit remaining on 160 panicles.)

Treatment	Weeks After Spraying 4	6	8	10	12	14	16	18
Unsp. Cont.	577	515	260	90	52	27	23	22
Sp-st. Cont.	468	341	125	35	15	10	9	9
H-4	573	435	205	58	28	17	17	17
H-5	479	417	208	64	30	13	11	9
M-4	658	615	359	134	74	36	27	21
M-5	515	425	224	61	33	19	16	16
N-4	755	639	363	125	71	47	40	38
N-5	530	423	219	84	35	18	16	16
O-4	435	364	173	50	29	15	14	13
O-5	662	573	265	88	33	8	5	4
Q-4	567	487	285	110	54	27	20	19
Q-5	670	558	349	119	61	20	16	15
Y-4	378	252	127	61	31	18	16	15
Y-5	589	502	254	80	48	24	22	21
Total	7,874	6,546	3,416	1,159	594	299	252	235

TABLE 6

COMPARISON OF INITIAL TWO COUNTS, 1960

(Numbers are total fruit remaining on 160 panicles.)

Treatments	First (T)	Second (r)	Ratio (r/T)	Rank	(r^2/T)
Unsp. Cont.	577	515	0.893	2	459.66
Sp-st. Cont.	468	341	0.729	13	248.46
H-4	573	435	0.759	12	330.24
H-5	479	417	0.871	3	363.03
M-4	658	615	0.935	1	574.81
M-5	515	425	0.825	9	350.73
N-4	755	639	0.846	7	540.82
N-5	530	423	0.798	11	337.60
O-4	453	364	0.804	10	292.49
O-5	662	573	0.866	4	495.97
Q-4	567	487	0.859	5	418.29
Q-5	670	558	0.833	8	464.72
Y-4	378	252	0.667	14	168.00
Y-5	589	502	0.852	6	427.85
Total	7,874	6,546			5,472.67
Minus correction factor = $\frac{(6,546)^2}{7,874}$			=		5,441.98
					30.69
Chi Square factor = $\frac{(7,874)^2}{(6,546)(1,328)}$			=		7.13

Chi Square = (30.69)(7.13) = 218.82

Chi Square for 13 D/F (0.001) = 34.528

Therefore, a very highly significant difference exists.

TABLE 7

SUMMARY OF ANALYSIS, INITIAL TWO COUNTS, 1960

(Chemicals Only)

Source	D/F	Sum of Squares	Chi Square Factor	Chi Square
Concentration	1	0.4458	7.1958	3.208
Chemicals	5	6.9648	7.1958	50.117
Conc x Chem	5	16.1517	7.1958	116.224
Total	11	23.5623		

	0.05	0.01	0.001
Chi Square for 1 D/F	3.841	6.635	10.827
Chi Square for 5 D/F	11.070	15.086	20.517

(The effect of concentration approaches but does not reach the 0.05 significance level. The effect of chemicals is very highly significant. Since the interaction (effect of concentration with chemical) is also very highly significant, no note can be taken of this chemical effect without further statistical analysis.)

TABLE 8

ANALYSIS OF THE BETTER CONCENTRATION OF EACH CHEMICAL (SECOND COUNT, 1960)

(Numbers are total fruit remaining on 160 panicles)

Treatments	First (T)	Second (r)	Ratio (r/T)	(r^2/T)
H-5	479	417	0.871	363.03
M-4	658	615	0.935	574.81
N-4	755	639	0.846	540.82
O-5	662	573	0.866	495.97
Q-4	567	487	0.859	418.29
Y-5	589	502	0.852	427.85
Total	3,710	3,233		2,820.77
Minus correction factor = $\frac{(3,233)^2}{3,710}$			=	2,817.33
				3.44
Chi Square factor = $\frac{(3,710)^2}{(3,233)(477)}$			=	8.93

Chi Square = (3.44)(893) = 30.63

Chi Square for 5 D/F (0.001) = 20.517

Therefore, a very highly significant difference exists.

TABLE 9

ANALYSIS OF THE POORER CONCENTRATION OF EACH CHEMICAL (SECOND COUNT, 1960)

(Numbers are total fruit remaining on 160 panicles.)

Treatments	First (T)	Second (r)	Ratio (r/T)	(r^2/T)
H-4	573	435	0.759	330.24
M-5	515	425	0.825	350.73
N-5	530	423	0.798	337.60
O-4	453	364	0.804	292.49
Q-5	670	558	0.823	464.72
Y-4	378	252	0.667	168.00
Total	3,119	2,457		1,943.78
Minus correction factor = $\frac{(2,457)^2}{3,119}$			=	1,935.51
				8.27
Chi Square factor = $\frac{(3,119)^2}{(2,459)(662)}$			=	5.98

Chi Square = (8.27)(598) = 49.45

Chi Square for 5 D/F (0.001) = 20.517

Therefore, a very highly significant difference exists.

TABLE 10

COMPARISON OF INITIAL AND FINAL COUNTS, 1960

(Numbers are total fruit remaining on 160 panicles.)

Treatments	First (T)	Final (h)	Ratio (h/T)	Rank	(h^2/T)
Unsp. Cont.	577	22	0.0381	3	0.8388
Sp-st. Cont.	468	9	0.0192	12	0.0173
H-4	573	17	0.0297	9	0.5044
H-5	479	9	0.0188	13	0.0169
M-4	658	21	0.0319	6	0.6702
M-5	515	16	0.0311	7	0.4971
N-4	755	38	0.0503	1	1.9126
N-5	530	16	0.0302	8	0.4830
O-4	453	13	0.0287	10	0.3731
O-5	662	4	0.0060	14	0.0242
Q-4	567	19	0.0335	5	0.6367
Q-5	670	15	0.0224	11	0.3358
Y-4	378	15	0.0397	2	0.5952
Y-5	589	21	0.0357	4	0.7487
Total	7,874	235			7.6540
Minus correction factor = $\frac{(235)^2}{7,874}$			=		7.0136
					0.6404
Chi Square factor = $\frac{(7,874)^2}{(235)(7,639)}$			=		34.5371

Chi Square = (0.6404) (34.5371) = 22.1176

Chi Square for 13 D/F (0.05) = 22.362

Therefore, no significant difference exists.

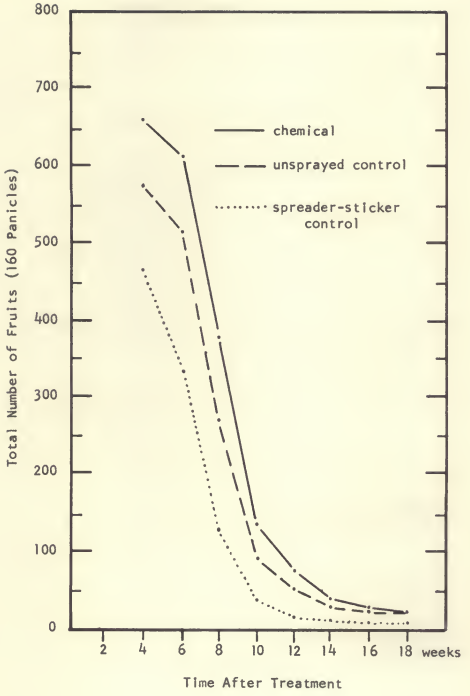

Figure 1. The effect of alpha-naphthaleneacetonitrile at 1 x 10^{-4} molar concentration on the fruit-set of mango.

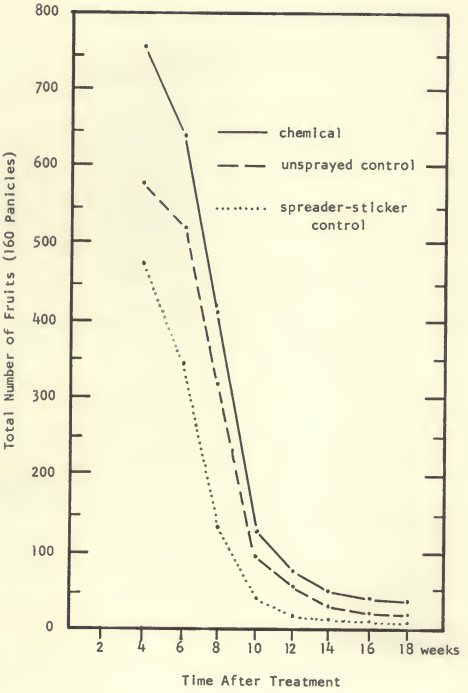

Figure 2. The effect of beta-naphthoxyacetic acid at 1 x 10^{-4} molar concentration on the fruit-set of mango.

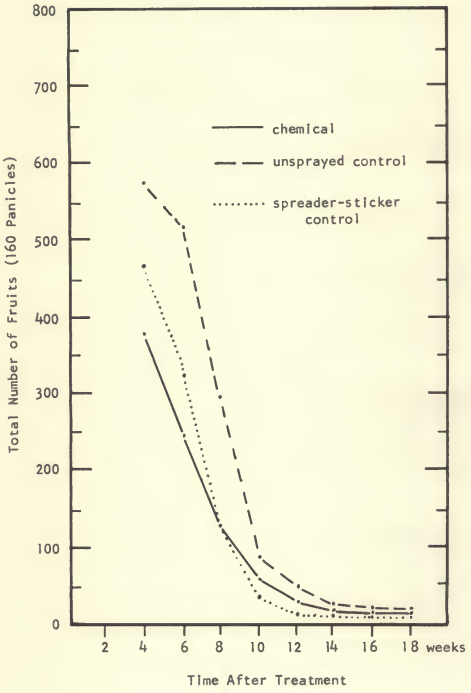

Figure 3. The effect of potassium gibberellate at 1 x 10^{-4} molar concentration on the fruit-set of mango.

Examination of the graphs (Figures 1, 2, and 3) shows that during the early counts there was a much greater difference among the readings than during the later counts. This observation is borne out by the analyses. The comparison of the initial two counts, as shown in Table 5, reveals that there is a very highly significant difference among the various treatments at this point. A detailed analysis, as shown in Table 7, indicates that there is a very highly significant interaction between the chemicals and the concentrations. This means that the chemicals do not act in the same way for both concentrations (1×10^{-4} molar and 1×10^{-5} molar). A very highly significant difference is also shown among chemicals. In view of this interaction no conclusions can be drawn about the effect of the chemicals alone without further statistical analysis.

This analysis was done by taking the better concentration for each chemical and comparing these among themselves (Table 8). Likewise the poorer concentrations for each chemical were compared among themselves (Table 9). Each of these analyses showed that there was a very highly significant difference among the effect of the chemicals in each group. This indicates that the chemicals had a marked effect on fruit retention at the second counting period (six weeks after treatment) as compared with the first counting period (four weeks after treatment).

Discussion

From the counts of fruits on these various treatments it is obvious that there is a definite and rapid loss of fruit from all treatments as well as from the controls. The rate of loss is practically the same for all treatments (as can be seen from the graphs).

From further examination of the graphs it is apparent that at harvest (18 weeks after treatment) there is very little difference in total numbers of fruits on the various treatments. This is confirmed by statistical analysis of the results at this point (Table 10). In this analysis the final counts were compared with the initial counts. This comparison shows that while the differences among the treatments approach the 0.05 significance level no marked difference existed at this point among the treatments.

Note is also made of the difference between the unsprayed control and the spreader-sticker control. From the data in Table 5 and from the graphs (Figures 1, 2, and 3) it can be seen that the unsprayed control had a consistently higher fruit-set than did the spreader-sticker control. The question then arises whether or not the spreader-sticker had an adverse effect on fruit-set. Since the spreader-sticker was also included with each chemical tested, it is then conceivable that with the spreader-sticker having this adverse effect, the chemicals would have to counteract this effect before a positive effect could be shown by them. If this was the case, then without the spreader-sticker, the chemicals which showed only a slight positive effect might possibly have shown a much greater positive effect.

From the rank of treatments on Table 6, it was shown that only one treatment (M-4) ranked better at the second count than did the unsprayed control. This relatively high ranking of the unsprayed control could possibly have been due to an adverse effect of the spreader-sticker. Nevertheless, a very highly significant difference existed among the treatments themselves which indicated that the applied growth substances had an effect on fruit retention, whether it was beneficial or harmful.

This same DuPont Spreader Sticker was used on the entire grove in conjunction with the regular fungicidal spray once a week during the months of January and February. The unsprayed control trees as well as the spreader-sticker control trees and all the chemical sprayed trees received these same fungicidal sprays. If the spreader-sticker had an adverse effect with this one added spray, it would seem possible that the fruiting of the entire grove would have been reduced. This same spreader-sticker has been used for a number of years on a good many crops with no obvious detrimental effect. For these reasons this possibility is discounted.

While a very highly significant difference existed among the treatments in both the 1959 screening tests and the 1960 spraying experiment, those substances used in both years did not rank in the same order each year. There are several reasons why this might be true. While efforts were made to have as many conditions the same for both experiments, there were a number of uncontrollable environmental conditions which undoubtedly had an effect on the results of these tests. In the 1959 tests three concentrations of each compound

were used (10^{-3} M, 10^{-4} M, and 10^{-5} M). In interpreting the results of these tests the counts from these three concentrations were grouped together. In the 1960 spraying experiment only two concentrations (10^{-4} M and 10^{-5} M) of each compound were used. In interpreting these results each concentration was examined. A very highly significant interaction between concentrations and chemicals was found to be present in 1960. Interaction was not statistically tested in 1959, but based on the 1960 results it would be logical to assume that some interaction was also present in 1959. In 1959 a solution of Tide detergent was used as the wetting agent, while in 1960 a commercial spreader-sticker (DuPont) was used for this purpose. Finally, the 1960 experiment was designed statistically to be more precise to correct some of the shortcomings of the 1959 tests. In the analysis of these results, sources of error (tree effect, interaction, etc.) were removed before considering the effect of the chemicals. Since there are these differences between the two years' experiments, it is not unreasonable to accept these differences in ranking as being realistic.

A comparison of the results of the 1959 screening test and the 1960 experiment are presented in graph form (Figure 4). It is interesting to note that the graphs of the unsprayed controls for both years and of the N-4 treatment (beta-Naphthoxyacetic acid, 1×10^{-4} molar concentration) were similar. The principal difference which can be seen in the results of the two years' experiments is that the 1959 counts showed a sharper decline in fruit-sets between the counts made six weeks and eight weeks after treatment. This confirmed the observations made in 1959 that much fruit was removed by wind. What was

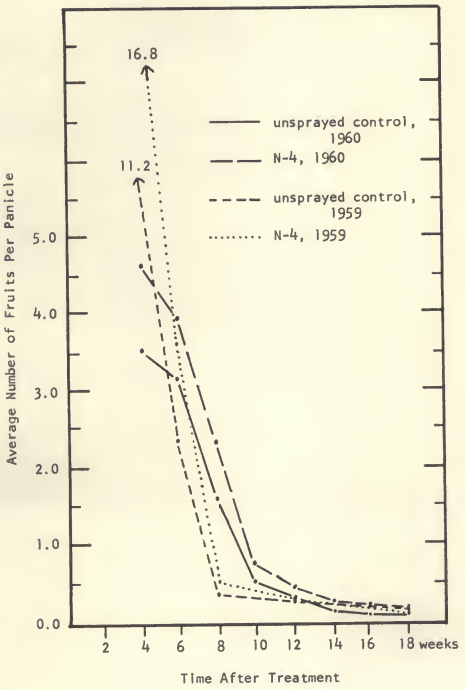

Figure 4. Comparison of results of 1959 and 1960 spray experiments on mango fruit-set showing unsprayed controls and N-4 treatment.

interesting in this regard was that even with this heavy reduction in fruits by wind, the harvest counts (18 weeks after treatment) were practically the same for all cases. From this it was apparent that while the wind did remove a number of fruit, these fruit would have fallen under natural conditions before maturity.

Summary and Conclusions

From the results of this experiment it is obvious that the various treatments had a highly significant effect on fruit retention during the early period of development. Some of the treatments (as exemplified by Figures 1 and 2) served to increase the numbers of fruit above those on the unsprayed controls, while others (as exemplified by Figure 3) served to reduce the numbers of fruits below those on the unsprayed controls. During the final stages of development, these differences ceased to exist and the counts of fruit on the various treatments and controls were practically the same.

The indications are, therefore, that some of these treatments do have a beneficial effect on the initial fruit-set of mango. As indicated by the data presented here, this beneficial effect is short-lived.

Because of this short effect, which did not last until maturity, it is necessary to investigate by further experimentation the advisability of applying repeated sprays at intervals during the early development period of the fruit. Further experimental work as to times of applications and concentrations of sprays, would have to be conducted. Experiments as to the advisability of including a spreader-sticker with these sprays are also indicated.

CHAPTER V

COLLATERAL TESTS

In addition to the main spraying experiment (Chapters III and IV) several collateral tests were conducted during the 1960 blooming and fruiting season. These were: (1) tip-feeding, (2) panicle pruning, (3) additional screening of chemicals for effect on mango fruit-set, and (4) weekly repeat spraying. These tests are discussed individually in this chapter.

Tip-Feeding

The basic assumption was made that poor fruit-set in monoembryonic varieties of mango may result from the abortion of embryos due to a lack of nutrients or growth factors. To test this assumption, additional nutrients and growth factors were supplied to the blossom panicle by tip-feeding.

Materials and Methods

A wide range of materials was used in this experiment so as to include as many sources as possible of some of the better known growth factors as well as nutrients. The materials used were as follows: casein hydrolysate, dried 95 per cent methonal extract of brewer's yeast diluted with one ml. (1:1) or four ml. (1:4) of water for each gram of original yeast, sucrose, coconut milk, and distilled water.

Three ml. of each substance were placed in 10 ml. test tubes. The ends of the blossom panicles were stripped of laterals back far enough to insert the rachis to the bottom of the tubes. After the rachis was inserted in the tube, the tube was plugged with cotton and secured in place. Control panicles were pruned but had no tube treatment. The time for tip-feeding was 48 hours.

It was desired to have the treated panicles under similar environmental conditions. Therefore, panicles for this test were selected from the same side of the tree and at approximately the same height. Enough panicles with these limitations were not available on one tree, so two trees were selected and the treatments were divided between them. Five panicles were used per treatment.

Results

The results of this tip-feeding test are presented in Table II. As can be seen, the casein hydrolysate and yeast extract caused an initial fruit-set greater than that of the controls. However, as with the spray treatments (Chapters III and IV), this initial increase in fruit-set disappeared toward maturity. On 50 panicles only three fruits remained at harvest (18 weeks after treatment). Of these three fruits, two were on control panicles.

Discussion

Since the yeast extract and the casein hydrolysate both contain a broad range of growth factors, it is not known which of these factors was responsible for this initial increase in fruit-set. These treatments could have supplied factors which either: (1) increased

TABLE 11

THE EFFECT OF TIP-FEEDING MANGO PANICLES
ON THE NUMBER OF FRUITS SET

(Numbers are total fruits on five panicles.)

Treatments	Weeks After Treatment							
	4	6	8	10	12	14	16	18
On Tree No. 2								
Casein hydrolysate (1.0%)	9	8	6	2	2	1	1	1
Casein hydrolysate (0.3%)	12	12	8	1	0	0	0	0
Casein hydrolysate (0.1%)	5	5	1	1	1	0	0	0
Coconut milk	3	3	2	1	0	0	0	0
Control	5	5	2	2	1	1	1	1
On Tree No. 4								
Distilled water	8	8	7	3	2	1	0	0
Sucrose (1×10^{-2} M)	12	10	7	0	0	0	0	0
Yeast extract (1:1)	27	21	12	3	0	0	0	0
Yeast extract (1:4)	43	32	18	2	1	0	0	0
Control	25	23	17	6	2	1	1	1
Total	149	127	80	21	9	4	3	3

initial fruit-set which later decreased, or (2) prolonged the eventual drop of the young fruits which did set, later leaving no noticeable increase in fruits at maturity.

Conclusions

From these data it is concluded that an initial increase in fruit-set was accomplished by tip-feeding mango blossom panicles with solutions of casein hydrolysate and yeast. The initial increase disappeared before maturity.

Panicle Pruning

The panicle-pruning test was conducted for the purpose of determining whether or not too many blossoms were competing for the existing nutrients available to a blossom panicle. Since there are several thousand blossoms per average panicle, it was desired to check on this competition factor.

Materials and Methods

Five panicles were treated in each of the following manners: (1) panicle cut in half, (2) only basal laterals left (rachis cut above basal "whorl"), (3) only terminal buds left, and (4) control (no pruning done).

All panicles were on the same tree and at the same height and exposure. The panicles were pruned and tagged for future recording of fruit-set.

Results

The results from this panicle-pruning test are recorded in Table 12. From the first count (four weeks after treatment), the control panicles had much higher fruit-set (with one exception--panicles cut in half had a slightly higher count at ten weeks after treatment). By the time of the fifth count (12 weeks after treatment) the only fruit left in the entire test was on a control panicle.

Discussion

This was a small exploratory test designed to probe the possibility that competition between blossoms might be a factor in poor fruit-set. It is conceivable that an experiment with many more replications might produce different results. However, on the basis of these exploratory results this possibility appears remote.

These results are in keeping with those of Young (78) on a similar test when he concluded removing blossoms from a panicle only reduced the number of potential fruits.

Conclusions

From these data it does not appear that a useful result is obtained from pruning the mango blossom panicle. On this basis it is assumed that the competition for nutrients between blossoms is probably not a major factor in poor fruit-set.

TABLE 12

THE EFFECT OF PRUNING MANGO PANICLES
ON THE NUMBER OF FRUITS SET

(Numbers are total fruits on five panicles.)

Treat-ments	Weeks After Treatment							
	4	6	8	10	12	14	16	18
Control	33	28	12	1	1	1	1	1
Cut in half	5	5	4	2	0	0	0	0
Only base left	1	1	1	0	0	0	0	0
Only tip left	9	9	2	0	0	0	0	0
Total	48	43	19	3	1	1	1	1

Additional Screening of Chemicals for Effect on Mango Fruit-Set

During the 1959 screening test (Chapter III) several chemicals did not respond as well as expected on the basis of their behaviour with other fruits. In addition, several other chemicals were tested for their effect on mango fruit-set.

Materials and Methods

The materials used in this screening test are listed in Table 13. (Those which are repeated from the 1959 test have the same code designation.)

The same procedure was used in this screening as was used in the 1959 screening test. The compounds were dissolved, pH adjusted to 7.0, and the volume brought up to 25 ml. by using a 0.05 per cent Tide solution which had previously been adjusted to pH 7.0. By series dilutions 25 ml. of 1×10^{-4} molar and 1×10^{-5} molar solutions were prepared. These solutions were applied to the blossom panicles with a glass atomizer. As in the 1959 test these concentrations were coded "-3," "-4," and "-5" respectively and used in combination with the compound code to give a combination compound and concentration code designation.

As in 1959, five panicles were used per treatment. A basic difference in method from the 1959 test was that the five panicles were on different trees. Five trees were used, and each tree had one representative of each treatment. This was done so that the tree effect could be statistically removed from consideration.

TABLE 13

LIST OF CHEMICALS USED, 1960 SCREENING

Code	Compound
G	Indoleacetic acid
K	1-Naphthaleneacetamide
L	alpha-Naphthaleneacetic acid
Z	Kinetin
DD	A.E.T. (Abbott)
EE	Thiourea
FF	Cysteine
GG	Adenine
HH	Guanine
II	Xanthine
JJ	Uracil

As controls, one panicle on each tree was sprayed with the 0.05 per cent Tide solution, and one unsprayed panicle per tree was marked for comparison purposes.

Results

The results from the 1960 test are recorded in Table 14. The first two counts (four and six weeks after treatment) reveal a good number of fruits set. By the time of the third count (eight weeks after treatment) the number of fruits remaining on the test panicles had dropped to a fairly low number.

Statistical analyses of the first two counts are recorded in Tables 15 and 16. After the second count when the totals dropped to a very low number, these analyses were discontinued. Examination of the results of these analyses of the first two counts shows that a highly significant difference exists among the chemicals in their effect on initial mango fruit-set.

At 16 weeks after treatment no further decline in fruit-set was evident over the 14-week count. By this time the fruit-set on five panicles had reached a point that the highest number was one. It is obvious that by this stage in the development of the fruit there was no difference among the treatments. At maturity (18 weeks after treatment) there was only a decline of one fruit from the previous count.

TABLE 14

RESULTS OF 1960 ADDITIONAL SCREENING TEST

(Numbers are total fruit on five panicles.)

Treatments	Weeks After Treatment							
	4	6	8	10	12	14	16	18
G-5	16	10	5	1	1	1	1	1
G-4	20	16	4	0	0	0	0	0
G-3	21	19	5	0	0	0	0	0
K-5	35	27	7	0	0	0	0	0
K-4	19	13	4	1	1	1	1	1
K-3	11	9	1	0	0	0	0	0
L-5	12	10	3	2	2	1	1	1
L-4	10	9	3	1	1	1	1	1
L-3	5	5	1	0	0	0	0	0
Z-5	14	9	2	0	0	0	0	0
Z-4	18	13	3	0	0	0	0	0
Z-3	10	6	1	0	0	0	0	0
DD-5	18	12	6	1	0	0	0	0
DD-4	17	11	4	2	1	1	1	1
DD-3	15	11	4	2	1	1	1	1
EE-5	23	17	2	1	0	0	0	0
EE-4	23	19	4	0	0	0	0	0
EE-3	15	12	7	1	0	0	0	0
FF-5	23	19	6	1	0	0	0	0
FF-4	21	20	9	1	1	0	0	0
FF-3	11	9	5	0	0	0	0	0
GG-5	10	9	3	0	0	0	0	0
GG-4	12	8	5	3	3	1	1	1
GG-3	19	17	4	0	0	0	0	0
HH-5	8	9*	7	2	2	1	1	1
HH-4	9	9	5	2	0	0	0	0
HH-3	12	4	3	1	0	0	0	0
II-5	11	11	2	0	0	0	0	0
II-4	9	12*	5	2	1	1	1	1
II-3	13	8	4	2	0	0	0	0
JJ-5	7	10*	6	1	1	1	1	1
JJ-4	7	3	1	1	0	0	0	0
JJ-3	12	11	2	0	0	0	0	0
TIDE	10	4	3	2	2	1	1	0
Unsp. C.	14	14	6	1	1	1	1	1
Total	510	405	142	31	18	12	12	11

(*In several cases some unopened flowers were present at the first count--four weeks after treatment. These were not counted as set fruit. Some of these developed into set fruit during the next two weeks and consequently show an increase in number of set fruit in several cases for the second count over the first count.)

TABLE 15

SUMMARY OF ANALYSIS, FIRST COUNT 1960
ADDITIONAL SCREENING TEST

Source	D/F	Sum Squares	Mean Square
Trees	4	86.4285	21.6071
Treatments:			
Level Linear	1	9.9000	9.9000
Level Quadratic	1	0.2455	0.2455
Chemicals	10	135.1758	13.5176**
Chem x Level L.	10	91.0000	9.1000*
Chem x Level Q.	10	14.5878	1.4588
Tide vs Unsp. C.	1	1.6000	1.6000
Conts. vs Treat.	1	2.8052	2.8052
(Total Treatments	34	255.3143)	
Error	136	621.9715	4.5733
Total	174	963.7143	

** = Highly significant (beyond 0.01 level)

* = Significant (beyond 0.05 level)

TABLE 16

SUMMARY OF ANALYSIS, SECOND COUNT 1960
ADDITIONAL SCREENING TEST

Source	D/F	Sum Squares	Mean Square
Trees	4	86.4285	21.6071
Treatments:			
Level Linear	1	9.9000	9.9000
Level Quadratic	1	0.2455	0.2455
Chemicals	10	135.1758	13.5176**
Chem x Level L.	10	91.0000	9.1000*
Chem x Level Q.	10	14.5878	1.4588
Tide vs Unsp. C.	1	1.6000	1.6000
Conts. vs Treat.	1	2.8052	2.8052
(Total Treatments	34	255.3143)	
Error	136	621.9715	4.5733
Total	174	963.7143	

** = Highly significant (beyond 0.01 level)

* = Significant (beyond 0.05 level)

Discussion

Here as in the previous tests (1959 screening test, 1960 full tree spray experiment, and the tip-feeding test) there was an initial difference among the various treatments which was negated as the development period of the fruit progressed. Work done by Luckwill (34, 35, 26) indicated a need at different times during the development of the fruit for more hormones than at other times to keep the fruit from abscissing. A similar situation is suggested by these results with the mango. Since these treatments were applied only once while the trees were in blossom, the possibility also presented itself that there might be a continuing need for these hormones during the entire development period of the fruit. These possibilities were used for the basis of the weekly repeat-spraying test (to be discussed next).

Conclusions

On the basis of these results, it is concluded that none of these tested compounds had an effect on improving fruit-set which would last until maturity. The chance that there is an effect for a limited time is realized.

Weekly Repeat-Spraying

Realizing that a single spray (as in the screening experiments) might not last throughout the entire development period of the fruit, it was decided to conduct a limited test with sprays on a repeated basis. The purpose of this test was to determine if a continual supply of growth substances available for the blossom and young fruit during the development period would increase fruit-set.

Materials and Methods

Without the background of a trial test the previous year, it was not known whether repeated sprays would increase or decrease fruit-set. In addition to a fruit-set response, damage to the tree might result. Because of this and of a shortage of trees, it was decided to conduct this test on a limited preliminary basis. One tree was used for each treatment, and only one concentration (1×10^{-4} molar) of each of the compounds was used. The chemicals and their code designations are:

Code	Chemical
H	3-Indoleacetic acid, ethyl ester
M	alpha-Naphthaleneacetonitrile
N	beta-Naphthoxyacetic acid
O	Phenoxyacetic acid
Q	gamma-Phenyl, N-butyric acid
Y	Potassium gibberellate

Each tree was sprayed completely once a week to a point of run-off. Forty panicles were tagged as in the full-tree spraying experiment--ten in each quadrant, three of which were in the upper third, four in the middle third, and three in the lower third. Counts of remaining fruits on each panicle were taken at two-week intervals, starting four weeks after the first spray. Sprays were applied on each of the following dates: January 27, February 3, 11, 18, and 25.

Results

The results from this weekly repeat-spraying test are recorded in Table 17. After the first count (four weeks after the first treatment--at the time of the fifth treatment) it was noted that the number of fruits remaining on the panicles was much lower than on the trees with only one spraying (Table 5). Therefore, this weekly repetition was discontinued.

TABLE 17

THE EFFECT OF REPEATED APPLICATIONS OF VARIOUS SPRAYS AT WEEKLY INTERVALS ON THE FRUIT-SET ON MANGO

(Numbers are total fruit on 40 panicles.)

Treat-ments	Weeks After Treatment 4	6	8	10	12	14	16	18
H-4	70	62	30	6	5	3	3	3
O-4	121	88	33	3	2	2	2	2
Q-4	64	52	23	3	2	1	1	1
M-4	88	74	30	10	5	5	5	5
N-4	77	59	33	6	2	1	1	1
Y-4	55	43	23	5	5	5	5	5
Control	112	94	38	6	3	2	2	2
Total	587	472	210	39	24	19	19	19

Discussion

It can only be conjectured that the repetition of these treatments reduced the yields. The possibility exists that the fruit was thinned by these treatments as is done in horticultural practice on several orchard fruits (e.g., apples and peaches). In some cases the same compound applied at different concentrations and at different times may be used for either fruit-setting or fruit-thinning (33). Therefore, the chance is not eliminated that sprays of weaker concentrations or applied at different intervals between treatments might have improved the yields.

Conclusions

The only conclusion which can be drawn from this test is that these particular sprays at these concentrations and applied at these intervals did apparently reduce the yields of fruit. Further experiments at different concentrations and different intervals will have to be carried out to determine whether or not this type of treatment might actually be an effective means of increasing the fruit-set of mango.

CHAPTER VI

CYTOLOGICAL OBSERVATIONS

As the chemical spraying experiment (Chapter IV) progressed throughout the fruit development period it became evident that the compounds used in the test were not increasing mango fruit-set to any appreciable degree over that of the unsprayed control. The basic assumption had been made that fertilization had occurred and that fruit-drop was due to an insufficient amount of hormones being supplied by the ovarian wall to prevent abscission of the young fruit until a developing embryo could supply these hormone requirements. If this was true in the mango, as it has been shown to occur with other fruits (34, 35), these treatments with growth substances should have shown a greater effect on fruit-set.

Cytological examinations were carried out, therefore, to determine if fertilization had occurred and whether or not this basic assumption was valid. To accomplish this, a comparison was made between the number of chromosomes in the endosperm cells and in the somatic cells of the nucellus.

Materials and Methods

Ovaries from a monoembryonic variety (Edward) were selected for examination which showed enlargement from flower size. They were killed and fixed in a mixture of glacial acetic acid and absolute

alcohol (1:3) and dehydrated through the tertiary butyl alcohol series (22). After imbedding in paraffin, serial sections were cut at 10 and 15 microns. Various stains were used including: (1) iron-alum haematoxylin and safranin, (2) safranin and fast green, (3) Martius yellow and lacmoid, and (4) resorcin blue.

Preparations of whole pistils squashed between two slides were stained with either Martius yellow-lacmoid mixture or with resorcin blue to determine the extent of pollen tube growth.

A comparison was made between a poor fruiting monoembryonic variety (Edward) and a heavy fruiting polyembryonic variety (Earle) to determine if there was a difference in their chromosome complement. Material which had been frozen fresh and stored in a frozen condition (originally collected for biochemical analyses--Chapter VII) was used for chromosome study. Two sizes of small fruits, approximately one-quarter and one half inch in length, of each variety were used. The ovules were removed and immediately placed in the glacial acetic acid-absolute alcohol mixture (1:3) overnight for killing and fixing.

The embryo sacs were dissected from the ovules, using the pectinase enzyme method of Forbes (11), stained with alcoholic aceto-carmine, and squashed under a cover glass. In the small ovaries the entire embryo sacs were squashed whole. In the large ovaries, which had too dense internal material for proper examination, the embryo sacs were opened and the contents spread carefully with dissecting needles.

Nucellar material from both varieties was removed by careful dissection, stained with alcoholic aceto-carmine and used to determine the somatic chromosome number (2n) of the two varieties.

Results

In the small ovaries of the monoembryonic variety, the egg (or fertilized zygote which had not divided) was evident in all but a very few of the series which were examined. No traces of the antipodals were found at this stage of development. In some of the ovaries one or both synergids were present, usually in a state of deterioration. In all examined ovaries less than 1.8 mm. in length, no endosperm was present although the endosperm nucleus was evident near the egg. In all examined ovaries 1.8 mm. in length or longer a multi-nucleate endosperm was found.

While germinating pollen grains were in evidence on the stigma of the monoembryonic variety, in no case was the pollen tube found to extend more than two or three diameters of the pollen grain down the style, which was approximately 1.5 mm. in length. In several of these cases where pollen tube growth was limited to the upper portion of the style, a multi-nucleate endosperm was already evident within the embryo sac. No divisions of the egg were evident at this stage.

The somatic chromosome number (2n) of the polyembryonic variety (Earle) was 40, as determined by counting the chromosomes in the cells of the nucellus and the nucellar embryos. This diploid count of 40 chromosomes is in keeping with the same number as determined by Mukherjee (46) for several species of Mangifera.

The chromosome number in the endosperm cells of the polyembryonic variety was found to be 40, the same as for the somatic cells. This is a diploid (2n) number instead of a triploid (3n) number such as would result from triple fusion. Thus, it would seem that fertilization had not occurred.

The somatic chromosome number (2n) of the monoembryonic variety (Edward) was also found to be 40 as determined by counting the chromosomes in the cells of the nucellus. An exact count was not obtained for the chromosomes in the endosperm of this variety; however, there appeared to be more chromosomes present than the diploid 40 as found in the polyembryonic variety. An estimation was made that this number was approximately 60, and thus it would seem that it was in a triploid (3n) condition.

Discussion

The development of the mango embryo sac was found to follow quite closely that outlined by Juliano (23, 24) and Juliano and Cuevas (25). Beiling (4) reported that all ovaries of the No. 11 variety greater than 7 mm. in length had a multi-nucleate endosperm while the majority of those less than 7 mm. in length had no multi-nucleate endosperm.

At first thought one might assume that the presence of a multi-nucleate endosperm is indirect evidence that fertilization, or at least triple fusion, had occurred. However, this condition might be the result of apomictic development (21, 43) resulting without fertilization.

It can be seen from these results that endosperm development and ovary enlargement can occur without the pollen tube reaching the embryo sac. However, this does not entirely preclude the occurrence of fertilization and triple fusion. Since only a few of the fruits which initially set are maintained until maturity, it is possible that in

the monoembryonic variety only those which have been fertilized and initiated a gametic embryo are those which remain. The other fruits which dropped as development progressed could conceivably have started due to apomictic endosperm development, but abscission occurred due to lack of embryo formation either from the egg or nucellus cells. In the polyembryonic variety a similar situation could then exist, except that in this case nucellar embryos developed which would then supply the necessary growth factors to maintain the fruit development until maturity.

Young (78) reported tracing the pollen tube of hand pollinated flowers to the micropyle, but did not report the entrance of the tube into the embryo sac. Maheshwari (42) indicated in 1934 that actual fertilization of the mango had never been observed. The fact that fertilization does occur in this fruit is confirmed by the many hybrids developed from crosses of known varieties. Since the percentage of fertilized fruits is apparently very low, the chances of finding actual fertilization occurring is also low.

From these chromosome counts it was clear that the polyembryonic variety could develop endosperm without triple fusion. This plus its nucellar embryos could very easily allow it to set fruit with viable seed without fertilization having occurred. Juliano (23, 24) and Juliano and Cuevas (25) working with polyembryonic varieties in the Philippine Islands reported that in many cases there were no gametic embryos formed, all embryos were nucellar. This could easily have been explained by these observations that polyembryonic varieties could set fruit with viable seed without fertilization.

In the monoembryonic variety checked, the indications were that fertilization and triple fusion, giving rise to a triploid (3n) endosperm, were necessary for viable seed formation. Since no nucellar embryos were formed, a developing gametic embryo would have been essential for seed formation. Triple fusion could then be associated with this normal fertilization.

Conclusions

From these results, it would seem that fertilization had not occurred before the ovaries started to enlarge. Endosperm development was found to be apomictic. Fruit enlargement was associated with endosperm development.

It was concluded that fertilization was not necessary for endosperm development and ovary enlargement. Also, it was determined from this examination that the polyembryonic variety (Earle) could set fruit with a diploid (2n) endosperm in place of the usual triploid (3n) endosperm. Thus, it would appear that this polyembryonic fruit could be set without fertilization.

In the case of the monoembryonic variety (Edward), an initial enlargement of the ovary could possibly have been due to development of the endosperm. For this fruit to develop to maturity, the indications were that formation of the gametic embryo by fertilization was essential.

CHAPTER VII

BIOCHEMICAL ANALYSES

Observations of bearing trees and previous trees (Chapter VI) have indicated that a difference exists between monoembryonic and polyembryonic varieties of mangos in regard to their fruit-setting ability. As shown in the cytological study (Chapter VI), an apparent morphological difference occurred between the two types of mangos. It was suggested that the ability of the polyembryonic varieties to initiate endosperm growth without fertilization, which in turn would influence a few somatic cells to behave as zygotes, could start a chain of events that resulted in fruit-set and development. Therefore, it was reasoned that there may exist a similarity in physiology between this morphological response and that of parthenocarpic fruiting. Thus, these tests were conducted for the purpose of trying to determine if there are any apparent differences in the growth-regulator content in the developing fruits of the two types which might help to explain this difference in bearing.

Materials and Methods

Samples of young fruit (approximately 3 to 10 mm. in length) were collected from a monoembryonic variety (Edward) and a polyembryonic variety (Earle). These fruits were frozen to approximately -30° C. immediately upon picking, transported in dry ice, and

stored in a deep freeze until analyzed. The material was gathered from unsprayed trees in the same grove in which the main spray experiment was conducted (at West Palm Beach, Florida). The analyses were carried out in the biochemical laboratory of the Department of Fruit Crops, Florida Agricultural Experiment Station, Gainesville, Florida.

The whole ovaries were ground in a Potter's homogenizer using chilled methanol as an extracting solvent. Three steepings of 30 minutes each were made with the entire apparatus kept cold in an ice bath. The solutions were decanted and filtered through a Whatman No. 1 filter paper. All the washings from a particular tissue were combined and evaporated to dryness under vacuum with the aid of a Rotovac. These dried extracts were kept refrigerated until further treated.

Extracts were separated by column chromatography using silicic acid as a matrix and various combinations of petroleum ether and n-butanol as eluting solvents. The technique of Powell (52) was used with slight modifications. Sixteen grams of silicic acid (100-200 mesh) were used as a bed in a column with 2 cm. inside diameter, resulting in a 7.5 cm. column. Redistilled petroleum ether and redistilled n-butanol were each saturated with 0.5 M formic acid in glass-distilled water and used as stock solutions. The eluting solvents were prepared from these stock solutions as follows:

Solvent	Composition (per cent)	Vol. Used (ml.)	Indole Derivatives Eluted (52)	Rf
A	99.8 petroleum ether 0.2 n-butanol	200	Ethyl indoleacetate Indoleacetonitrile	.97 .99
B	97.0 petroleum ether 3.0 n-butanol	200	Indolebutyric acid Indolepropionic acid Indoleacetic acid	.56 .44 .37
C	75.0 petroleum ether 25.0 n-butanol	100	Indoleacetamide	--
D	25.0 petroleum ether 75.0 n-butanol	100	Tryptophan Tryptamine	.19 .75
E	100.0 n-butanol	200	---	
F	100.0 0.5 M formic acid	100	---	

The extracts were transferred to the column with the aid of a small amount of hydrated silicic acid and a portion of solvent A. At the beginning of each solvent change a small portion of the next solvent was used to rinse the sample container. Each solvent mixture (A, B, C, D, E, or F) was collected in a single container. Each fraction was evaporated to dryness under vacuum with the aid of a Rotovac and stored in a refrigerator until examined further.

The residue from each extraction was taken up in a small amount of methanol and spotted on separate 3 MM Whatman chromatographic paper strips which were 4 cm. wide. Fractions were divided and spotted on two strips: one was used for a bio-assay and the other was used for a chemical test. The strips were placed in a "Chromatocab" and left to equilibrate for 16 hours with the vapors from the solvent system. Following equilibration, the strips were developed by an ascending solvent system of Ispropanol (80): water (19): ammonia (1).

Development time was 20 hours. After air-drying, the chromatograms were examined under ultraviolet light (2,600 Angstrom units) and notes were made of the resulting colors.

One set of chromatographs was sprayed with Erlich's reagent (1 per cent p-dimethylaminobenzaldehyde in 1 N HCl). Note was made of the color reactions immediately after spraying and again after 24 hours.

The other set of chromatographs was analyzed by Avena bio-assay for growth-regulator activities. The Avena coleoptile straight-growth test was used as recommended by Nitsch and Nitsch (47). Each chromatogram was cut into 20 parts, each representing one-half an Rf zone. The resulting promotion and inhibition of growth in relation to a control was plotted on a histogram. The histograms for corresponding fractions of the two tissues were compared.

Results

The results from the various tests are presented in the following tables. Table 18 contains a comparison of the polyembryonic and monoembryonic varieties with a listing of the various growth promotors as revealed by the Avena bio-assay, together with the colors as noted under white light and ultraviolet light and after spraying with Erlich's reagent. It can be seen that five different growth promotors were found in the polyembryonic extracts while only one was found in the monoembryonic extracts. The one found in the monoembryonic extract (solvent E, Rf 0.2-0.25) was of a similar Rf and color reaction to one found in the polyembryonic extracts. It is entirely possible that other promotors, similar to those found in the polyembryonic extracts, might

TABLE 18

GROWTH PROMOTORS FOUND IN MANGO

Polyembryonic Variety (Earle)

Solvent	Avena Growth Test (Rf)	White Light	Ultraviolet Light	Erlich's Reagent
A	0.0 - 0.05 **	light brown	tan-gray	yellow
	0.2 - 0.3 *	light brown	grayish	yellow
	0.6 - 0.85 *	light brown	white	---
B	---			
C	0.5 - 0.65 **	light yellow	purple	yellow
D	---			
E	0.05 - 0.45***	dark brown	brown	yellow-brown
F	---			

Monoembryonic Variety (Edward)

Solvent	Avena Growth Test (Rf)	White Light	Ultraviolet Light	Erlich's Reagent
A	---			
B	---			
C	---			
D	---			
E	0.2 - 0.25 *	dark brown	dark brown	yellow-brown
F	---			

* significant beyond 0.05 level
** significant beyond 0.01 level
*** significant beyond 0.001 level

have also been present in the monoembryonic extracts but in such a low concentration that they could not be detected with these tests. However, since several more quantitative and qualitative promotors can be detected in the polyembryonic extracts than in the monoembryonic extracts, there appear to be more promotion factors present in the former than the latter. This would suggest that one reason why the polyembryonic varieties might be better fruiters than are the monoembryonic varieties is the presence of a larger quantity of growth-promoting factors which could better support growth and continued development of the fruits.

Table 19 contains a similar listing of growth inhibitors with their characteristics when analyzed by the Avena bio-assay and under white and ultraviolet light. A broad range of growth inhibitors was found in both types of extracts. There were more inhibitors to a greater intensity and spread found in the monoembryonic fractions than in the polyembryonic fractions. This is the opposite situation than is found for the growth promotors (Table 18). However, here again the polyembryonic varieties may have a better chance of development of the fruits since they have less inhibitors present.

Other tests were run with similar results. Since different solvent systems were employed, they cannot be directly compared in these tables.

Discussion

There appears to be a difference in growth-regulator content between the monoembryonic and polyembryonic types of mangos. While

TABLE 19

GROWTH INHIBITORS FOUND IN MANGO

Polyembryonic Variety (Earle)

Solvent	Avena Growth Test (Rf)	White Light	Ultraviolet Light	Erlich's Reagent
A	---			
B	0.65 - 0.75 *	light green	pink	light tan
C	0.05 - 0.50 *	light brown	gray	light brown
	0.65 - 0.75 **	---	---	---
D	0.00 - 0.80 ***	brown	brown	yellow-brown
E	0.45 - 0.85 ***	yellow streaks on brown	white	yellow-brown
F	0.40 - 0.70 **	---	---	---

Monoembryonic Variety (Edward)

Solvent	Avena Growth Test (Rf)	White Light	Ultraviolet Light	Erlich's Reagent
A	0.65 - 0.90 **	---	white	light tan
B	0.05 - 0.10 *	light green	dark purple	---
	0.15 - 0.55 *	light green	red - pink	---
	0.60 - 0.95 *	light green	light tan	---
C	0.60 - 0.80 **	light brown	white	yellow
D	0.00 - 0.85 ***	dark brown	brown	yellow-brown
E	0.35 - 0.80 ***	gray-green and yellow	dark brown	yellow-brown
F	---			

* significant beyond 0.05 level
** significant beyond 0.01 level
*** significant beyond 0.001 level

these quantitative differences appear to exist, it cannot at this stage of experimentation be determined whether this difference is due to cause or effect. That is, it is not known if these differences are due to the presence of several embryos per ovule in the polyembryonic variety as compared with the single embryo per ovule in the monoembryonic variety, or whether they might be due to some qualitative difference in the endosperm or other parts of the ovules. However, no matter where the growth promotors arise, it would appear that the polyembryonic fruits can develop to maturity since they are present.

The growth promotors and inhibitors here found present are themselves interesting since several would seem to be different from other known growth regulators found in plant materials (52). A comparison of the growth promotors (Table 18) with indole derivatives eluted (52, page 72) shows little correlation between the two. In the A fraction three promotors were found. The two with the lower Rf values are far below either ethyl indoleacetate or indoleacetonitrile. The third with the higher Rf value (0.6 - 0.85) could possibly be one of those with a higher Rf value as indicated on page 70. The one promotor found in the C fraction could coincide with the indoleacetamide found in the C fraction by Powell (52). The strong promotor (0.2 - 0.25 Rf) found in the E fraction is specially interesting as no corresponding promotors with these characteristics have previously been reported. The isolation and identification of these substances will have to be accomplished by further experimentations.

The results of the over-all tree-spray experiment (Chapter IV) and the screening test (Chapters III and V) indicated that there was an

early effect on initial fruit-set of mango due to some of the various growth substances tried. This effect disappeared later. The identification of the actual growth substance present in the better fruiting types (polyembryonic) would give an indication as to the best chemical substances to use in an attempt to induce fruit-set. The growth substances found at various stages in the developing fruit could then be applied at comparable stages in the development of the poorer fruiting types (monoembryonic).

Since a morphological difference was found to exist between these types of mangos, it is not known whether these applications would increase fruit-set at maturity (which is the ultimate objective of this experiment) or merely have an initial effect as experienced with the chemical growth substances used in these tests (Chapters III, IV, and V). Provided that the proper growth substance could be found and the proper concentration and time of application be determined, at best this would only be an artificial means of increasing fruit-set.

On the other hand, the combining through breeding of the desired morphological characteristics of the better-fruiting varieties (polyembryonic) with the more desirable physical characteristics (size, color, and flavor) of the poorer-fruiting varieties (monoembryonic) might be in the long run a more practical solution to this whole mango fruit-set problem. If a natural internal means was bred into a good quality fruit to increase its fruit-set, this would be a very desirable accomplishment.

Summary and Conclusions

From the results of this experiment, five growth promotors were found in the polyembryonic variety while only one was found in the monoembryonic variety. In contrast, six growth inhibitors were found in the polyembryonic variety while seven were found in the monoembryonic variety.

From this evidence that more growth promotors are present in polyembryonic mangos and more growth inhibitors are present in monoembryonic mangos, it is concluded that this could possibly explain the difference in fruit-set observed between these two types. The presence of growth promotors in the polyembryonic variety could stimulate the growth and development of the fruits. In the case of the monoembryonic variety, the presence of growth inhibitors could be the factor which results in an aborted gametic embryo and as a consequence the premature loss of fruit.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

Summary

Twenty-six chemical growth regulators were used on a monoembryonic variety (Edward) of mango during the 1959 season to try to increase fruit-set. Some of these showed an increase in initial fruit-set, but no apparent difference persisted until maturity. From these 26 compounds, six were selected to use on a larger scale the following year based upon their behavior in the preliminary test. In this enlarged experiment similar results were obtained, that is, an initial improvement in fruit-set was noted which did not continue until harvest.

During the 1960 season 11 different substances were screened on a limited scale with the objective to determine if any of these would significantly increase fruit-set more than those used in 1959. Here similar results were obtained in that some of the compounds had an early effect which did not persist while others had a detrimental effect.

Tests with pruning panicles and increasing available nutrients by tip-feeding were also tried in 1960. The object of these tests was to determine if competition between existing flowers or lack of nutrients within the panicle was affecting fruit-set. No increase in fruit-set was experienced with panicle pruning. Tip-feeding with casein hydrolysate and yeast extracts gave an initial increase in fruit-set which did not last until maturity.

When tests were run with repeated sprays on a weekly basis the resulting fruit-set was less than on unsprayed trees. This test was designed to make the growth substances available to the fruit for a longer period during their development. However, improper timing of applications could have thinned the fruits, nullifying any positive effect.

The developing fruits were examined cytologically to determine whether fertilization and/or triple fusion had occurred. A comparison was also made in this regard between a heavy-fruiting polyembryonic variety (Earle) and a poor-fruiting monoembryonic variety (Edward). No evidence was found that either fertilization or triple fusion had occurred in the polyembryonic variety. However, endosperms were found to exist in a diploid (2n) condition in place of the previously assumed triploid (3n) condition. This showed that an endosperm developed without triple fusion, which, in conjunction with nucellar embryos (polyembryony), could result in apomictic fruit-set of this type of mango.

The indications are that the endosperm of the monoembryonic variety was of the triploid (3n) condition, which necessitated triple fusion. Since no nucellar embryos exist in this type, fertilization was required for fruit-set.

Biochemical analyses were conducted to determine whether this morphological difference existing between the monoembryonic and polyembryonic varieties could be correlated in any way with the growth-regulator content of the developing fruit. The polyembryonic variety was found to contain five growth promotors while the monoembryonic

variety contained only one. The polyembryonic variety was found to contain six growth inhibitors in contrast to seven found in the monoembryonic variety.

Conclusions

1. No increase in fruit-set of the mango was obtained at maturity by spraying with various growth substances at time of bloom.

2. An effect on initial fruit-set of a very highly significant nature was obtained by treatments with various chemical growth substances. However, this effect was transitory and at maturity no difference among the various treatments persisted.

3. Sprays of growth substances applied at weekly intervals to the mango decreased the fruit-set as compared to unsprayed controls at the same time of fruit development.

4. Reduction of the number of flowers on a panicle does not increase fruit-set by lessening the competition among blossoms.

5. Supplying certain nutrients and other growth factors to the panicles by tip-feeding gave an initial increase in fruit-set which did not persist until maturity.

6. The polyembryonic variety tested had an endosperm with a diploid ($2n = 40$) chromosome complement, showing that triple fusion had not occurred.

7. The indications are that the monoembryonic variety tested had a triploid ($3n = 60$) endosperm, suggesting that triple fusion had occurred.

8. The assumption was made that the polyembryonic variety is able to set fruit without fertilization because of its apomictic endosperm plus its nucellar embryo development.

9. The assumption was made that the monoembryonic variety must have fertilization and triple fusion occurring in order to have fruit-set.

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BIOGRAPHICAL SKETCH

Thomas Tracy Sturrock was born in Havana, Cuba, on December 9, 1921, while his parents were temporarily in that country. He received his grammar school and high school education in the public schools of West Palm Beach, Florida, graduating from Palm Beach High School in 1939. He graduated from Palm Beach Junior College in 1941, and from the University of Florida with a Bachelor of Science in Agriculture with Honors in February 1943 and a Master of Science in Agriculture in August 1943.

He served with the U.S. Army Air Corps from August 1943 until November 1946. He has remained in the reserve and has recently been selected for promotion to Lt. Colonel in the U.S. Air Force Reserve.

Upon release from military duty he entered grove maintenance and fruit nursery business in West Palm Beach, Florida. He served as an inspector with the State Plant Board of Florida for seven months in 1956 and 1957 during the Mediterranean Fruit Fly eradication campaign. For a year and a half in 1957 and 1958 he taught biology and general science in Palm Beach High School. In 1958 he returned to the University of Florida for further graduate work.

On June 30, 1948, he was married to Jeanne Norquist of Boise, Idaho. They are the parents of five children.

The author is a member of Phi Kappa Phi, Alpha Zeta, and Phi Sigma. In addition he holds memberships in Florida Mango Forum, Florida State Horticultural Society, and American Society for Horticultural Science.

The research for this Doctor of Philosophy degree was completed in the summer of 1960. At the present time he is a member of the faculty of Palm Beach Junior College.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

January 28, 1961

W. A. Brooker
Dean, College of Agriculture

Dean, Graduate School

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